Synthesis of a Small Molecule Combinatorial Library Encoded with Molecular Tags

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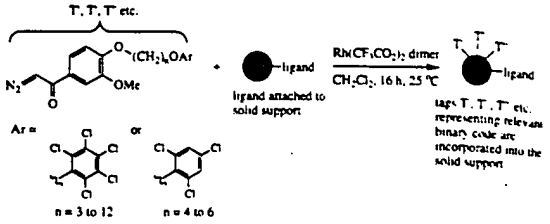
Currently there is considerable interest in using small molecule combinatorial libraries to accelerate the drug discovery process.[1] Combinatorial libraries have the potential to provide large numbers of novel compounds for random screening, supplementing the traditional sources of chemical collections and natural products. They can also facilitate the drug optimization process which follows lead identification through a more focused library design.

The preferred approach to combinatorial library construction on solid support is via the Furka split synthesis method.[2−8] Such libraries are usually prepared in a nonencoded form, in which selected compounds are identified either by microsequencing or by deconvolution strategies.[2−4] However, neither of these identification methods is readily applicable to nonsequencable molecules in large libraries. Several encoding strategies have recently been described to overcome this major obstacle.[5−8] In these approaches, each set of reagents in every synthetic step is encoded by separate entities attached either to the ligand itself or the solid support. At the end of the synthesis, each bead incorporates a single ligand, together with a unique set of tags describing its synthetic history. Several encoding technologies ave recently been developed, including the use of molecular,[5] oligonucleotide,[6] and peptide tags.[7,8] Of these, the molecular tags are the most chemically inert and are therefore capable of withstanding the varied reaction conditions needed to assemble a wide range of ligand families. They also have the added advantages of being routinely and rapidly detectable in trace amounts in a format amenable to automation and do not require complex protection/deprotection strategies.

The synthesis of the first small molecule combinatorial library encoded with molecular tags is described here. In this first example, a number of important issues were addressed and successfully resolved, including (i) the ability to synthesize small molecules encoded predictably on solid phase; (ii) the removal of library members from the solid support for biological testing in solution-based assays; (iii) the orthogonal detachment of the molecular tags from beads which provided biologically active ligands, with subsequent decoding to give the ligand structures; and (iv) confirmation of structural and biological data of active molecules.

Scheme 1. Encoding of Resin with Molecular Tags by Carbene Insertion



The library construction incorporated three combinatorial steps, utilizing sets of seven, 31, and 31 moieties, to produce a total of 6727 unique members (7 × 31 × 31 = 6727). The first combinatorial step used seven moieties, including four *N*-Fmoc amino alcohols and three *N*-Fmoc amino acids. These were attached to amino-functionalized poly(ethylene glycol)-grafted polystyrene resin via a photocleavable linker (*vide infra*).[9] The amino acids were directly attached to linker-derivatized resin via esterification, and the amino alcohols were initially attached to the photocleavable linker by carbonate formation in solution and then bound to the resin by amide formation. Each of the seven resin samples was then encoded with a unique combination of three molecular tags based on the binary code (*vide infra*). Prior to the second combinatorial step, all seven resin batches were pooled and the Fmoc protecting groups removed. The resin was then split into 31 equal batches, and 31 *N*-Fmoc amino acids (with acid labile protecting groups on side chains, where necessary) were coupled via amide bond formation. Each of the 31 resin samples was then uniquely binary encoded with a distinct set of five molecular tags. All resin batches were again pooled, the Fmoc protecting groups removed, and the resin split a final time into 31 batches for the third combinatorial step. The 31 reagents used included functionalized sulfonyl chlorides, isocyanates, carboxylic acids, and chloroformates, producing sulfonamides, ureas, amides, and carbamate linkages, respectively. Binary encoding was completed with a further distinct set of five molecular tags. Final pooling of the resin was followed by removal of any acid labile protecting groups. The completed library of 6727 members can be represented by all possible permutations of $R_1-R_2-R_3$ as shown in Chart 1.

Encoding of the library after each combinatorial step was accomplished by rhodium-catalyzed carbene insertion of diazomethyl ketone precursors of molecular tags attached to an oxidatively labile linker directly into the bead resin (Scheme 1).[5b] This carbene insertion of the linker/tag into the resin matrix is versatile and facile and does not require any special functionality typically required by all other encoding systems. As stated above, a binary encoding scheme was utilized, and encoding after combinatorial steps one, two, and three was performed with distinct sets of three, five, and five molecular tags, respectively. Ultimately, the synthetic history of each ligand was identified by a unique code consisting of the presence or absence of 13 molecular tags.[10]

The photocleavable linker utilized in the construction of this library facilitated a controlled release of ligand by exposure to

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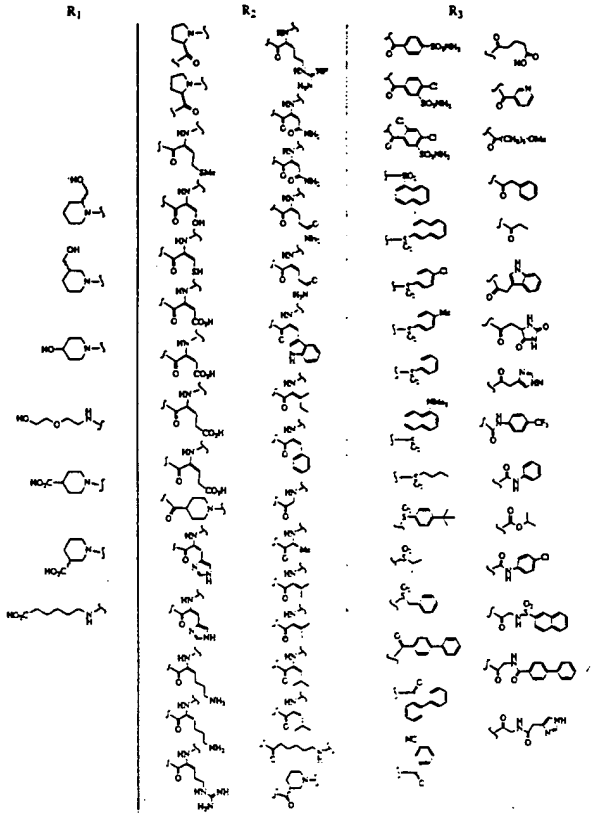
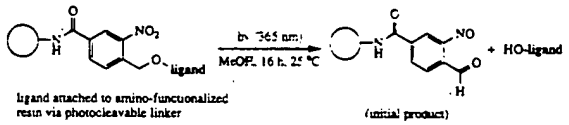
Chart 1. Library of 6727 Members Represented by All Permutations of $R_1-R_2-R_3$



Scheme 2. Photolytic Cleavage of Ligand from Resin



long-wavelength UV light, allowing bioassays to be performed in solution and multiple determinations to be made from a single bead (Scheme 2).[9] Resin beads were arrayed, either individually or in groups, into 96 well microtiter plates, and the ligands were subsequently photoeluted prior to biological screening.

In order to bias subsets of members toward activity in several multitest assays, known pharmacophoric elements were incorporated into the library. These included the arylsulfonamide moiety, a known pharmacophore versus the enzyme carbonic anhydrase (CA).[11] Photoelution of ligands from the resin and screening versus human CAII provided novel inhibitors with K_i values covering a wide potency range.[12] A number of these compounds were subsequently resynthesized in solution, and structural and biological analysis confirmed the results obtained directly from the library.

Release of tags from beads which produced active ligands and subsequent decoding was accomplished by oxidative

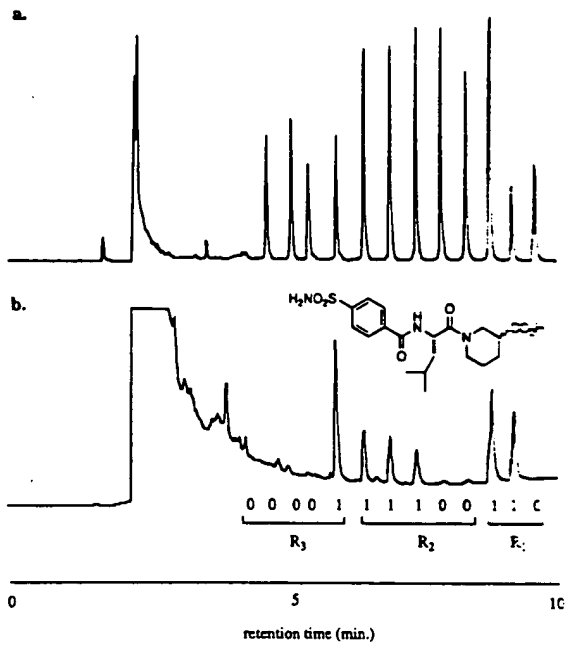
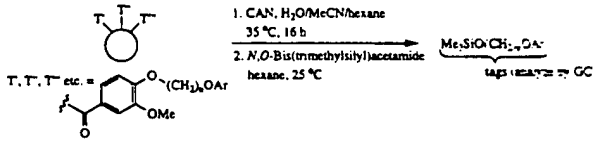


Figure 1. (a) Standard ECGC chromatogram showing the set of molecular tags used to encode library synthesis. (b) Representative encoding chromatogram from a bead identified in a carbonic anhydrase screen with the corresponding decoded structure.

Scheme 3. Oxidative Release and Subsequent Decoding of Molecular Tags



removal using ceric ammonium nitrate (CAN), silylation, and analysis by electron capture gas chromatography (ECGC)[13] (Scheme 3). Routinely, ~0.1 pmol of each tag is removed from a bead for analysis. The standard chromatogram, together with a representative decoded ECGC chromatogram, is shown in Figure 1.

In summary, a 6727 member small molecule encoded combinatorial library was synthesized on solid phase, each member attached to the resin by a photocleavable linker and uniquely identified by a binary code established using 13 molecular tags. Studies are now in progress to expand the repertoire of organic reactions compatible with solid phase synthesis and to apply this binary encoding technology to produce other small molecule combinatorial libraries.

Supplementary Material Available: Full experimental details for the synthesis and encoding of the library, together with procedures for the photolytic release of ligands, the CA assay, and subsequent decoding of pertinent beads; the solution syntheses of four human CAII inhibitors are also included (21 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(10) Thirteen tags should permit up to $2^{13} = 8192$ compounds to be distinguished. However, the codes of each synthetic step represented by no tags are not utilized. This precaution acts as a quality control in the encoding technology, such that at least one tag from each coding group should always be detected upon decoding. For this particular library, this allows $(8 - 1) \times (32 - 1) \times (32 - 1) = 6727$ ligands to be encoded.

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